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(57) Abstract

A rapid and quantitative method for producing a sterile, injectable solution of Tc-99m-labeled monovalent antibody fragment comprises the step of mixing a sterile solution containing a monovalent antibody fragment having at least one free sulfhydryl group, stannous chloride and excess tartrate, at mildly acidic pH, or a sucrose-stabilized lyophilizate of such solution, with a sterile solution of Tc-99m-pertechnetate, whereby substantially quantitative labeling of the antibody fragment with Tc-99m is effected in about 5 minutes at ambient temperature, the resultant sterile solution of Tc-99m-labeled monovalent antibody fragment being suitable for immediate injection into a patient for radioimmunodetection.

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METHOD FOR RAPIDLY RADIOLABELING MONOVALENT ANTIBODY FRAGMENTS WITH TECHNETIUM

Background of the Invention

The present invention relates to a method and kit for directly and rapidly radiolabeling a monovalent antibody fragment with technetium-99m (Tc-99m), using one or more pendant sulfhydryl groups as endogenous ligands, and more particularly to a method and kit for radiolabeling Fab or Fab' antibody fragments to prepare a sterile, Tc-99m-labeled antibody fragment solution which is almost immediately ready for injection into a patient for radioimmunodetection.

Prior art methods for binding Tc-99m ions directly to antibodies and antibody fragments are discussed in U.S. Patent Application Serial Nos. 07/176,421, 07/364,373, and 07/392,280. Those applications also disclose and claim improved methods for effecting direct radiolabeling of antibodies and antibody fragments with various radioisotopes, including Tc-99m and Re-186/188.

European Patent Application A2/0 237 150, to NeoRx Corp., and PCT Application WO 88/07382, to Centocor Cardiovascular Imaging Partners, L.P., each disclose methods for radiolabeling an antibody or antibody fragment with Tc-99m, but the labeling conditions are not optimized for labeling Fab or Fab' fragments and the disclosed conditions are inconvenient and do not result in quantitative labeling.

A need continues to exist for a direct method for stably radiolabeling Fab and Fab' antibody fragments within a few minutes to produce an solution which is ready for immediate injection into a patient for scintigraphic imaging.

Objects of the Invention

Accordingly, it is a primary object of the present invention to provide a method for direct radiolabeling of a monovalent, e,g,. Fab or Fab', antibody fragment which is rapid and convenient and which results in a labeled fragment ready for direct injection into a patient.

Another object of the invention is to provide an "instant" Tc-99m labeling kit for labeling a Fab or Fab' antibody fragment that is stable to prolonged storage but that can be combined directly with the sterile saline effluent from a Tc-99m generator to produce a sterile solution of radioantibody fragment.

Upon further study of the specification and appended claims, further objects and advantages of this invention will become apparent to those skilled in the art.

Summary of the Invention

The foregoing objects are achieved by providing a method for producing a sterile, injectable solution of Tc-99m-labeled monovalent antibody fragment, which comprises the step of mixing:

(1A) a sterile solution containing a unit dose for scintigraphic imaging of a monovalent antibody fragment having at least one free sulfhydryl group, stannous chloride in an amount of about 10-150 μg Sn per mg of antibody fragment, and about a 30-40-fold molar excess of tartrate over stannous chloride, in about 0.04 - 0.06 M acetate buffer containing saline, at a pH of 4.5 - 5.0, or

(1B) the lyophilizate of a sterile solution containing a unit dose for scintigraphic imaging of a monovalent antibody fragment having at least one free sulfhydryl group, stannous chloride in an amount of about 10-150 μg Sn per mg of antibody fragment, and about a 30-40-fold molar excess of tartrate over stannous chloride, in about 0.04 - 0.06 M acetate buffer containing saline and made about 0.08 - 0.1 M in sucrose, at a pH of 4.5 - 5.0;

with (2) a sterile solution containing an effective scintigraphic imaging amount of Tc-99m-pertechnetate,

whereby substantially quantitative labeling of the antibody fragment with Tc-99m is effected in about 5 minutes at ambient temperature, the resultant sterile solution of Tc-99m-labeled monovalent antibody fragment being suitable for immediate injection into a patient for radioimmunodetection.

Kits for use in the foregoing method are also provided.

Detailed Description

The present inventors have significantly improved the reagents and conditions for a kit and method for "instant" labeling of monovalent, e.g., Fab or Fab', antibody fragments containing at least one and preferably a plurality of spatially adjacent stabilized free sulfhydryl groups. Labeling is effected substantially quantitatively at ambient temperature within about 5 minutes of mixing a solution of antibody fragment with pertechnetate, readily available from commercial generators.

Details regarding conventional reagents and procedures are found in the three parent applications incorporated by reference herein and are not reiterated herein.

It will be understood that the monovalent antibody fragments to be radiolabeled can be fragments which bind to antigens which include but are not limited to antigens produced by or associated with tumors, infectious lesions, microorganisms, parasites, myocardial infarctions, atherosclerotic plaque, or normal organs or tissues. It will also be understood that the term "monovalent antibody fragment" as used herein denotes Fab and Fab' fragments, normally obtained by cleavage of bivalent fragments or intact immunoglobulin. However, monovalent fragments can also include any fragments retaining the hypervariable, antigen-binding region of an immunoglobulin and having a size similar to or smaller than a Fab' fragment. This will include genetically engineered and/or recombinant proteins, whether single-chain or multiple-chain, which incorporate an antigen binding site and otherwise function in vivo as targeting vehicles in substantially the same way as natural immunoglobulin fragments.

Fab' antibody fragments are normally and conveniently made by reductive cleavage of $F(ab')_2$ fragments, which themselves are normally made by pepsin digestion of intact immunoglobulin. Cleavage is advantageously effected with thiol reducing agents, e.g., cysteine, mercaptoethanol, dithiothreitol (DTT), glutathione and the like. The cleaved $F(ab')_2$ fragment containing at least one free sulfhydryl group will be termed "Fab'-SH" herein. Fab antibody fragments are normally and

conveniently made by papain digestion of intact immunoglobulin, preferably in the presence of a thiol reducing agent. Cleaved $F(ab)_2$ will be termed "Fab-SH" herein.

Reduction of $F(ab')_2$ fragments is preferably effected at pH 5.5-7.5, preferably 6.0-7.0, more preferably 6.4-6.8, and most preferably at about pH 6.6, e.g., in citrate, acetate or phosphate buffer, preferably phosphate-buffered saline, and advantageously under an inert gas atmosphere. It is well known that thiol reduction can result in chain separation of the light and heavy chains of the fragment if care is not taken, and the reaction must be carefully controlled to avoid loss of integrity of the fragment.

Cysteine is preferred for such disulfide reductions and other thiols with similar oxidation potentials to cysteine will also be advantageously used. The ratio of disulfide reducing agent to protein is a function of interchain disulfide bond stabilities and must be optimized for each individual case. Cleavage of $F(ab')_2$ antibody fragments is advantageously effected with 10-30 mM cysteine, preferably about 20 mM, and a protein concentration of about 10 mg/ml.

Reduction of a $F(ab')_2$ fragment with known disulfide bond reducing agents gives after a short time, typically less than one hour, including purification, Fab' typically having 1-3 free sulfhydryl groups by analysis. Sulfhydryl groups can be introduced into an antibody fragment to improve radiometal binding. Use of Traut's Reagent (iminothiolane) for this purpose is not preferred, whereas use of oligopeptides containing several adjacent sulfhydryl groups is efficacious. In particular, use of metallothionein or, preferably, its C-terminal hexapeptide fragment (hereinafter, "MCTP"), is advantageous.

The Fab-SH or Fab'-SH fragments are advantageously then passed through a short sizing gel column which will trap low molecular weight species, including excess reducing agent. Suitable such sizing gel columns include, e.g., dextrans such as Sephadex G-25, G-50 (Pharmacia), Fractogel TSK HW55 (EM Science), polyacrylamides such as P-4, P-6 (BioRad), and the like. Cleavage can be monitored by, e.g., size exclusion HPLC, to adjust conditions so that Fab or Fab' fragments are produced to

an optimum extent, while minimizing light-heavy chain cleavage, which is generally less susceptible to disulfide cleavage.

The eluate from the sizing gel column is then stabilized in about 0.03 - 0.07, preferably about 0.05 M acetate buffer, pH about 4.5, made in about 0.1 - 0.3, preferably about 0.15 M saline, and preferably purged with an inert gas, e.g. argon. In general, it is advantageous to work with a concentration of antibody fragment of about 0.5 - 5 mg per ml, preferably about 1 - 3 mg/ml, of solution.

The stabilized Fab-SH or Fab'-SH fragments are next mixed with stannous ion, preferably stannous chloride, and with a stabilizer for the stannous ions. Stannous ion is readily available as its dihydrate, or it can be generated in situ from tin metal, e.g., foil, granules, powder, turnings and the like, by contact with aqueous acid, e.g., HCl. It is usually added in the form of $SnCl_2$, advantageously in a solution that is also about 0.01 N in HCl, in a ratio of about 10-150, preferably about 123 μg Sn per mg of fragment. Advantageously, the stannous ion solution is prepared by dissolving $SnCl \cdot 2\ H_2O$ in 6 N HCl and diluting the resultant solution with sterile H_2O that has been purged with argon.

A stabilizing agent for the stannous ion is advantageously present in the solution. It is known that ascorbate can improve specific loading of a chelator with reduced pertechnetate and minimize formation of TcO_2, when the reducing agent is stannous ion. Other polycarboxylic acids, e.g., tartrate, citrate, phthalate, iminodiacetate, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) and the like, can also be used. Although polycarboxylic acids are mentioned, by way of illustration, any of a variety of anionic and/or hydroxylic oxygen-containing species could serve this function, e.g., salicylates, acetylacetonates, hydroxyacids, catechols, glycols and other polyols, e.g., glucoheptonate, and the like. Preferred such stabilizers are ascorbate, citrate and tartrate, more preferably tartrate.

While the precise role of such agents is not known, it appears that they chelate stannous ion and may prevent adventitious reactions and/or promote reduction by stabilization of

stannic ions, and they may also chelate -- and thereby stabilize -- certain oxidation states of reduced pertechnetate, thereby serving as transchelating agents for the transfer of these technetium ions to the presumably more stable chelation with one or more thiol groups and other nearby ligands on the protein. Such agents will be referred to as "stabilizers" herein. The molar ratio of stabilizer to stannous ion is about 30:1 - 40:1.

A solution of stabilizer, e.g., NaK tartrate, advantageously at a concentration of about 0.1 M, in buffer, preferably sodium acetate, at a pH of about 5.5, is prepared with sterile H_2O purged with argon. One volume of the $SnCl_2$ solution is mixed with enough of the stabilizer solution to provide a 30 - 40 molar excess, relative to the stannous ion, and the resultant solution is sterile filtered and purged with argon.

The sterile, stabilized $SnCl_2$ solution is mixed with the sterile Fab'-SH or Fab-SH solution to obtain a final concentration of about 10-150, preferably about 123 μg Sn per mg of fragment. The pH is adjusted, if necessary to about 4.5 - 4.8.

The solution of fragment and stabilized stannous ion is advantageously metered into sterile vials, e.g., at a unit dosage of about 1.25 mg fragment/vial, and the vials are either stoppered, sealed and stored at low temperature, preferably in liquid nitrogen, or lyophilized. In the latter case, the solution is made about 0.09 molar with a sugar such as trehalose or sucrose, preferably sucrose, prior to metering into sterile vials. The material in the vials is then lyophilized, the vacuum is broken with an inert gas, preferably argon, and the vials containing the lyophilizate are stoppered, sealed and stored, optionally in the freezer. The lyophilization conditions are conventional and well known to the ordinary skilled artisan. Both the sealed lyophilizate and the sealed liquid nitrogen stored solution are stable for at least 9 months and retain their capacity to be rapidly and quantitatively labeled with Tc-99m ions upon mixing with pertechnetate.

To label a unit dose of antibody fragment, a vial of liquid nitrogen frozen solution is thawed to room temperature by gentle warming, or a vial of lyphilizate is brought to ambient temperature if necessary, and the seal is broken under inert gas,

preferably argon. A sterile saline solution of a suitable imaging quantity of pertechnetate is added to the vial and the contents are mixed. When labeling the foregoing unit dosage quantity of antibody fragment, the amount of pertechnetate is generally about 1 - 100 mCi/mg of antibody fragment, and the time of reaction is about 0.1 - 10 min. With the preferred concentrations of protein and stannous ions noted above, the amount of pertechnetate is preferably about 5 - 20 mCi/mg, and the time of reaction is preferably about 1 - 5 min. This is effectively an "instant" labeling procedure with respect to the prior art processes which generally required 30 minutes to several hours incubation, in some cases at elevated temperatures and/or with additional purification required.

Pertechnetate is generally obtained from a commercially available generator, most commonly in the form of $NaTcO_4$, normally in saline solution. Other forms of pertechnetate may be used, with appropriate modification of the procedure, as would be suggested by the supplier of a new form of generator or as would be apparent to the ordinary skilled artisan. Pertechnetate is generally used at an activity of about 0.2-20 mCi/ml in saline, e.g., 0.9% ("physiological") sterile saline, optionally buffered at a pH of about 3-7, preferably 3.5-5.5, more preferably about 4.5-5.0. Suitable buffers include, e.g., acetate, tartrate, citrate, phosphate and the like.

The process according to the present invention routinely results in substantantially quantitative incorporation of the label into the antibody fragment in a form which is highly stable to oxidation and resistant to transchelation in saline and serum. When labeled with Tc-99m according to the method of the present invention, 100% incorporation of Tc-99m to Fab' is seen (within the limits of detection of the analytical monitor) together with >95% retention of immunoreactivity. The radioantibody solutions as prepared above are ready for immediate injection, if done in a properly sterilized, pyrogen-free vial. Also, no blocking of free sulfhydryl groups after technetium binding is necessary for stabilization. Furthermore the immunoreactivity of the labeled fragment is hardly reduced after serum incubation for a day,

showing that the conjugates are still completely viable imaging agents out to at least 24 hours.

It will also be apparent to one of ordinary skill that the resultant Tc-99m-radiolabeled antibody fragments are suitable, and in fact particularly convenient and efficacious, in methods of non-invasive scintigraphic imaging of tumors and lesions. In particular, in a method of imaging a tumor, an infectious lesion, a microorganism, a parasite, a myocardial infarction, a clot, atherosclerotic plaque, or a normal organ or tissue, wherein an antibody fragment which specifically binds to an antigen produced by or associated with said tumor, infectious lesion, microorganism, parasite, myocardial infarction, clot, atherosclerotic plaque, or normal organ or tissue, and radiolabeled with a pharmaceutically inert radioisotope capable of external detection, is parenterally injected into a human patient and, after a sufficient time for the radiolabeled antibody or antibody fragment to localize and for non-target background to clear, the site or sites of accretion of the radiolabeled antibody fragment are detected by an external imaging camera, it will be an improvement to use as the radiolabeled antibody fragment a Tc-99m-labeled antibody fragment made according to the method of the present invention. Such imaging methods are well known in the art.

The labeled fragments are also useful for detecting tumors and lesions and defining their boundaries, in intraoperative or endoscopic detection modalities, according to well known methods, e.g., those disclosed in Martin, Jr., et al., U.S. Patent No. 4,782,840, or in Goldenberg, U.S. Patent Application Serial No. 06/943,561. The foregoing scintigraphic, intraoperative and endoscopic methods are all embraced by the term radioimmunodetection.

A kit for use in radiolabeling a monovalent antibody fragment, e.g., an Fab'-SH or Fab-SH fragment, with Tc-99m, using generator-produced pertechnetate, (illustrative of the generic kit as claimed herein, with variations that would be apparent to the ordinary skilled artisan) would typically include about 0.01 - 10 mg, preferably about 1 - 2 mg, per unit dose of an antibody fragment which specifically binds an antigen, e.g., an antigen

associated with a tumor, an infectious lesion, a microorganism, a parasite, a myocardial infarction, a clot, atherosclerotic plaque, or a normal organ or tissue, and which contains at least one but preferably a plurality of adjacent free sulfhydryl groups; about 10 - 150 μg per mg of fragment of stannous ions and a 30 - 40 molar excess, relative to the stannous ions, of a stabilizer such as tartrate. The constituents of the kit are provided in a single, sealed sterile vial, in the form of a solution or a lyophilizate, and are mixed just prior to use with about 2 - 100 mCi of Tc-99m pertechnetate per mg of antibody or antibody fragment. Normally, the kit is used and/or provided in combination with one or more auxiliary reagents, buffers, filters, vials, columns and the like for effecting the radiolabeling steps.

The foregoing are merely illustrative and many varients can be envisioned for use with the variations in the process of the invention described hereinabove.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. In the following examples, all temperatures are set forth uncorrected in degrees Celsius; unless otherwise indicated, all parts and percentages are by weight.

Example 1

Preparation of Tc-99m-anti-CEA-Fab'

A. Labeling Kit

The following solutions are prepared.

(I) A solution of 0.075 M $SnCl_2$ is prepared by dissolving 3350 mg $SnCl \cdot 2\ H_2O$ in 1 ml of 6 N HCl and diluting the resultant solution with sterile H_2O that has been purged with argon.

(II) A solution of 0.1 M NaK tartrate in 0.05 M NaAc, at pH 5.5, is prepared with sterile H_2O purged with argon.

(III) One volume of solution I is mixed with 26 volumes of solution II, and the resultant solution is sterile filtered and purged with argon.

(IV) A solution of anti-CEA-Fab'-SH, prepared from a murine monoclonal IgG_1 antibody that specifically binds to carcinoembryonic antigen (CEA) by pepsin cleavage to an $F(ab')_2$ fragment, is reduced to Fab'-SH with 20 mM cysteine; excess cysteine is removed by gel filtration, and the Fab'-SH is stabilized (2 mg/ml) at pH 4.5 in 0.05 M NaOAc buffer which is 0.15 M in saline; and the resultant solution is sterile filtered and purged with argon.

(V) Mix solution IV with enough of solution III to obtain a final concentration of 123 μg Sn per mg of Fab'-SH, and adjust the pH to 4.5 - 4.8.

Fill solution V, under argon, into sterile vials (1.25 mg Fab'-SH per vial), stopper, crimp-seal and store vials in liquid nitrogen.

Alternatively, make solution V 0.09 M with sucrose, fill the resultant solution, under argon, into sterile vials (1.25 mg Fab'-SH per vial) and lyophilize. Break the vacuum with argon, stopper the vials containing the lyophilizate and crimp-seal the vials.

B. Labeled Fragment

Gently warm a vial of liquid nitrogen stored fragment or select a vial of lyophilizate prepared according to part A above. Inject a sterile solution of 10 mCi of sodium pertechnetate in sterile saline from a generator into the vial of Fab'-SH and stabilized stannous ions and mix by gentle agitation. Labeling is quantitative in five minutes, and the resultant solution of Tc-99m-labeled fragment is ready for immediate injection into a patient.

Example 2

Tumor Imaging

A sterile solution of a unit dose of Tc-99m-labeled anti-CEA-Fab' prepared (with liquid nitrogen stored Fab'-SH solution) according to Example 1 is infused intravenously into a patient with a progressively rising CEA titer, the patient having undergone "curative" surgery for a colon carcinoma three years earlier. Scintigraphic imaging 2 hr postinjection demonstrates antibody fragment localization in the pelvis at the site of removal of the primary tumor. Subsequent surgery confirms the presence of a 1.0 x 0.5 cm carcinoma that is successfully removed.

Example 3

Tumor Imaging

A sterile solution of a unit dose of Tc-99m-labeled anti-CEA-Fab' prepared (from lyophilizate) according to Example 1 is infused intravenously into a patient with a 3 x 2 cm rectal polyp that has been proven by biopsy to be malignant. Imaging 2 hr postinjection demonstrates localized antibody fragment in the primary tumor, the right lobe of the liver and in the lower lobe of the left lung. Needle biopsy confirms the presence of tumor in both the liver and the lung. The original plan to perform surgery and adjuvant radiation therapy is abandoned and palliative chemotherapy is instituted.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

WHAT IS CLAIMED IS:

1. A method for producing a sterile, injectable solution of Tc-99m-labeled monovalent antibody fragment, which comprises the step of mixing:

(1A) a sterile solution containing a unit dose for scintigraphic imaging of a monovalent antibody fragment having at least one free sulfhydryl group, stannous chloride in an amount of about 10-150 μg Sn per mg of antibody fragment, and about a 30-40-fold molar excess of tartrate over stannous chloride, in about 0.04 - 0.06 M acetate buffer containing saline, at a pH of 4.5 - 5.0, or

(1B) the lyophilizate of a sterile solution containing a unit dose for scintigraphic imaging of a monovalent antibody fragment having at least one free sulfhydryl group, stannous chloride in an amount of about 10-150 μg Sn per mg of antibody fragment, and about a 30-40-fold molar excess of tartrate over stannous chloride, in about 0.04 - 0.06 M acetate buffer containing saline and made about 0.08 - 0.1 M in sucrose, at a pH of 4.5 - 5.0;

with (2) a sterile solution containing an effective scintigraphic imaging amount of Tc-99m-pertechnetate,

whereby substantially quantitative labeling of the antibody fragment with Tc-99m is effected in about 5 minutes at ambient temperature, the resultant sterile solution of Tc-99m-labeled monovalent antibody fragment being suitable for immediate injection into a patient for radioimmunodetection.

2. The method of claim 1, wherein said monovalent antibody fragment is a Fab-SH or Fab'-SH fragment.

3. The method of claim 1, wherein said antibody or antibody fragment specifically binds a tumor marker.

4. The method of claim 1, wherein said antibody or antibody fragment specifically binds an antigen associated with an infectious lesion, a microorganism or a parasite.

5. The method of claim 1, wherein said antibody or antibody fragment specifically binds an antigen associated with a myocardial infarction, a clot or atherosclerotic plaque.

6. The method of claim 1, wherein said antibody or antibody fragment specifically binds an antigen associated with a normal organ or tissue.

7. A kit suitable for radiolabeling a monovalent antibody fragment with Tc-99m, which comprises a sealed, sterile container containing a sterile solution consisting essentially of a unit dose for scintigraphic imaging of a monovalent antibody fragment having at least one free sulfhydryl group, stannous chloride in an amount of about 10-150 μg Sn per mg of antibody fragment, and about a 30-40-fold molar excess of tartrate over stannous chloride, in about 0.04 - 0.06 M acetate buffer containing saline, at a pH of 4.5 - 5.0; wherein said antibody fragment specifically binds to an antigen produced by or associated with a tumor, an infectious lesion, a microorganism, a parasite, a myocardial infarction, a clot, atherosclerotic plaque, or a normal organ or tissue.

8. A kit suitable for radiolabeling a monovalent antibody fragment with Tc-99m, which comprises a sealed, sterile container containing the lyphilizate of a sterile solution consisting essentially of a unit dose for scintigraphic imaging of a monovalent antibody fragment having at least one free sulfhydryl group, stannous chloride in an amount of about 10-150 μg Sn per mg of antibody fragment, and about a 30-40-fold molar excess of tartrate over stannous chloride, in about 0.04 - 0.06 M acetate buffer containing saline and made about 0.08 - 0.1 M in sucrose, at a pH of 4.5 - 5.0; wherein said antibody fragment specifically binds to an antigen produced by or associated with a tumor, an infectious lesion, a microorganism, a parasite, a myocardial infarction, a clot, atherosclerotic plaque, or a normal organ or tissue.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05196

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 43/00, 39/395: C07K 15/28

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	US: 424/1.1,424/85.91,530/389,390 and IPC(5): A61K 43/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

APS MESSENGER TEXT SEARCH

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US, A, 3,725,295 (ECKELMAN et al.) 03 April 1973, see column 2, lines 25-56 and column 3, line 69 bridging column 4, line 41.	
Y,P	US, A, 4,877, 868 (RENO ET AL.) 31 October 1989, see the entire document.	1-8
A	US, A, 4,057,617 (ABRAMOVICI ET AL.) 08 November 1977 see column 2, line 63 bridging column 3, line 42.	
A	US, A, 4,293,537 (WONG) 06 October 1981, see column 4, lines 1-35.	
A	US, A, 4,401,647 (KROHN ET AL.) 30 August 1983, see column 3, lines 8-47 and column 5, lines 13-29.	
A	US, A, 4,472,371 (BURCHIEL ET AL) 18 September 1984 see column 8, line 33 bridging column 9, line 19.	
Y	US, A, 4,478,815 (BURCHIEL ET AL.) 23 October 1984 see column 6, lines 37-39 and column 7, lines 11-68.	6-8
A	US, A, 4,500,507 (WONG) 19 February 1985 see oclumn 2, line 64 bridging column 3, line 59.	
A	US, A, 4,062,933 (WOLFANGEL ET AL) 13 December 1977 see column 3, line 12 bridging column 4, line 10.	

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
19 OCTOBER 1990	01 FEB 1991
International Searching Authority [1]	**Signature of Authorized Officer** [10]
ISA/US	FOR: NANCY-SO INTERNATIONAL DIVISION JOHN M. COVERT

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